Selected Reading

Adams, J.S., Chen, H., Chun, R., Gacad, M.A., Encinas, C., Ren, S., Nguyen, L., Wu, S., Hewison, M., and Barsony, J. (2004). J. Steroid Biochem. Mol. Biol. *89–90*, 461–465.

Gekle, M. (2005). Annu. Rev. Physiol. *67*, 573–594.

Griffin, J.E., Leshin, M., and Wilson, J.D. (1982). Am. J. Physiol. *243*, E81–E87.

Hammes, A., Willnow, T.E., Andreassen, T.K., Spoelgen, R., Raila, J., Metzger, J., Schweigert, F.J., Luppa, P.B., Nykjaer, A., Huebner, N., and Schultz, H. (2005). Cell *122*, this issue, 751–762.

Hijazi, R.A., and Cunningham, G.R. (2005). Annu. Rev. Med. *56*, 117–137.

Mendel, C.M. (1989). Endocr. Rev. *10*, 232–274.

Nagai, J., Christensen, E.I., Morris, S.M., Willnow, T.E., Cooper, J.A., and Nielsen, R. (2005). Am. J. Physiol. Renal Physiol. *289*, F569–F576.

Nykjaer, A., and Willnow, T.E. (2002). Trends Cell Biol. *12*, 273–280.

Nykjaer, A., Dragun, D., Walther, D., Vorum, H., Jacobsen, C., Herz, J., Melsen, F., Christensen, E.I., and Willnow, T.E. (1999). Cell *96*, 507–515.

Soccio, R.E., and Breslow, J.L. (2004). Ateriosclerosis. Thromb. Vasc. Biol. *24*, 1150–1160.

DOI 10.1016/j.cell.2005.08.024

The Intestinal Heme Transporter Revealed

The iron-containing porphyrin heme provides a rich source of dietary iron for mammals. The fact that animals can derive iron from heme implies the existence of a transporter that would transport heme from the gut lumen into intestinal epithelial cells. In this issue of *Cell*, Shayeghi, McKie, and coworkers (Shayeghi et al., 2005) now describe a heme transporter that is expressed in the apical region of epithelial cells in the mouse duodenum. Their identification of heme carrier protein 1 (HCP1) provides a major missing piece in our understanding of iron uptake and mammalian nutrition.

It had been established long ago that the duodenal mucosa is the site of dietary iron absorption in mammals. However, the identity of the putative iron transporters expressed by duodenal epithelial cells remained a mystery until the past decade. Using several techniques including expression cloning (Gunshin et al., 1997) and positional cloning in iron-deficient mouse and zebrafish models, researchers identified the apical and basolateral iron transporters of the duodenum: divalent metal transporter (DMT1) and ferroportin or Ireg 1, respectively (reviewed in Andrews, 2000). At the same time, McKie's group took a different approach that enabled them to potentially identify multiple duodenal iron transporters (McKie et al., 2000, 2001). Based on the observation that intestinal iron uptake is clearly regulated according to the iron needs of the animal, they focused their efforts on a strain of mouse with congenital hypotransferrinemia. In these mice, low levels of the iron binding protein transferrin results in profound anemia and a marked increase in intestinal iron uptake leading to tissue iron overload. These hypotransferrinemic mice develop anemia because the transferrin that carries ferric iron in the bloodstream is virtually the only source of iron for developing red blood cells (Ponka, 2002).

Reasoning that increased iron uptake in the hypotransferrinemic mice could be due to increased expression of iron transporters in gut epithelial cells, McKie and coworkers used RNA subtraction techniques to identify genes with increased expression patterns in the duodenum of hypotransferrinemic mice compared with wild-type animals. Subtraction libraries enable researchers to clone and study genes that are regulated under specific physiological conditions by isolating RNA transcripts that are differentially expressed. To highlight differential gene expression in the duodenum, they subtracted transcripts that were also expressed in the ileum, a portion of the intestine that does not absorb iron. From one subtraction library, they identified the basolateral iron exporter Ireg (McKie et al., 2000), also known as ferroportin (Donovan et al., 2000) or MTP1 (Abboud and Haile, 2000), and Dcytb, a highly regulated reductase of the intestinal apical mucosa (McKie et al., 2001). They also identified DMT1, which had already been characterized.

These studies set the stage for their new work (Shayeghi et al., 2005). McKie and colleagues surmised that there was still a major molecular player in the uptake of iron from the gut that had not yet been identified. It is well established that heme iron is an extremely efficient source of this element in the mammalian diet, supporting the notion that a dedicated and highly regulated transporter for iron derived from heme must be present in the duodenum (Wheby et al., 1970). In this issue, these investigators now describe the cloning and molecular characterization of a heme transporter expressed in the apical region of duodendal epithelial cells (Shayeghi et al., 2005). This transporter, which they call heme carrier protein 1 (HCP1), transports iron bound to heme from the gut lumen into duodenal epithelial cells; the iron is then released from heme and may be transported into the bloodstream by ferroportin. The identification of HCP1 is an important accomplishment that supplies a key missing piece in our understanding of iron uptake and mammalian nutrition.

HCP1 is highly conserved and is part of a larger family of transporters known as the major facilitator superfamily (MFS) (Abramson et al., 2004). Within this family, HCP1 most resembles a bacterial protein that transports the antibiotic tetracycline. Notably, there are similarities in structure between the planar heme ring and tetracycline-metal structures that must be transported across the apical membrane of gut mucosal cells. The source of energy that drives heme transport is not yet known, although it most likely involves cotransport of a proton or anion along a favorable concentration gradient, similar to the mechanism used by other MFS transporters (Abramson et al., 2004).

Although radiolabeled heme is technically challenging to work with because it sticks to membranes and causes a high background count, McKie and coworkers successfully demonstrated in carefully controlled experiments that HCP1 facilitates specific heme uptake.

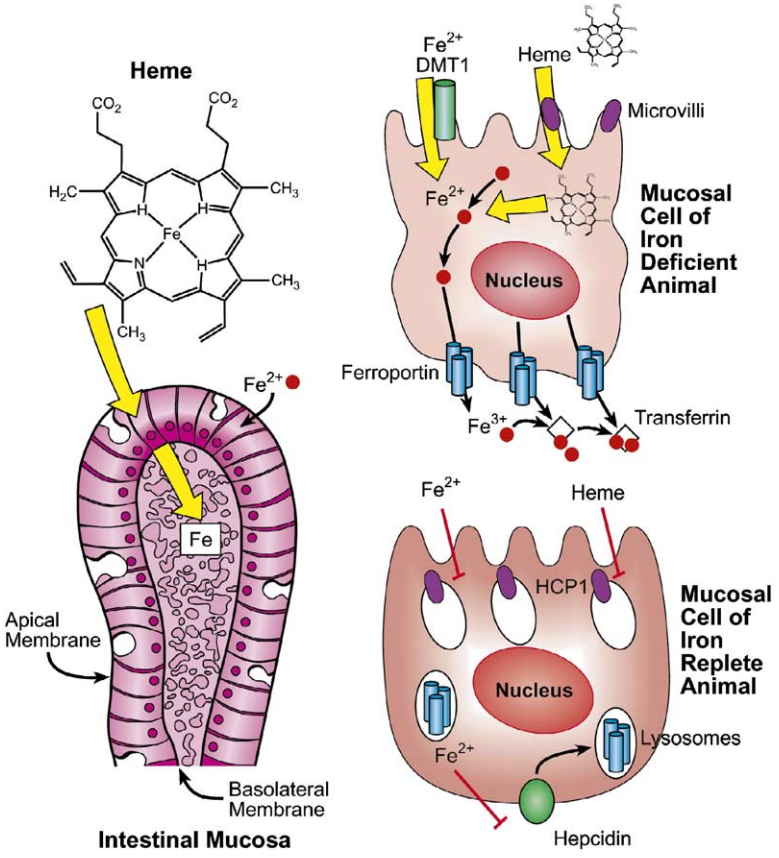

Figure 1. The Duodenal Mucosa of the Gut Is the Main Site of Iron Absorption in Mammals

(Left) Heme carrier protein (HCP1) is a transporter expressed in the apical membrane of duodenal epithelial cells that carries the porphyrin heme containing iron from the gut lumen into intestinal epithelial cells. (Right) HCP1 (purple) is expressed on the apical membrane of duodenal epithelial cells in iron-deficient mice, but, when the mice are iron replete, HCP1 relocates to intracellular compartments. The divalent metal transporter (DMT1) transports ferrous iron across the apical membrane of gut epithelial cells. Ferroportin exports iron across the basolateral membrane of gut epithelial cells into the bloodstream where it is carried in a bound state by the protein transferrin. In iron-deficient mice, there is an increase in the expression of genes that encode DMT1, Dcytb, and ferroportin in the duodenum. In iron-replete animals, the liver releases the peptide hormone hepcidin, which binds to ferroportin and causes its internalization and degradation in lysosomes.

Upon entering duodenal cells, heme most likely is degraded by the enzyme heme oxygenase, releasing ferrous iron and the bile pigment biliverdin. The iron released from heme is then transported across the epithelial cell basolateral membrane by ferroportin and is released into the bloodstream where it binds to its carrier transferrin. Interestingly, HCP1 also facilitates uptake of zinc protoporphyrin, which differs from heme only in that zinc is bound to the position within the porphyrin ring that iron occupies in heme. The cytosol of cells that produce HCP1 fluoresces when zinc protoporphyrin is added to their growth medium because zinc protoporphyrin naturally emits a fluorescent signal, providing a useful marker for zinc or free protoporphyrin.

Perhaps the most interesting aspects of the Shayeghi et al. paper relate to how HCP1 is regulated. The authors isolated HCP1 by subtraction procedures that depended on a difference in mRNA expression between hypotransferrinemic and wild-type mice. However, the changes in HCP1 expression that they detected in the duodenum of hypotransferrinemic mice were unexpectedly small. In addition, unlike the iron exporter Ireg 1 and the apical ferric reductase, Dcytb, the amount of HCP1 transcript did not increase in the duodenum of iron-deficient mice unless the mice were exposed to hypoxic conditions. Inspection of the promoter sequence for the *HCP1* gene did not reveal binding sites for the hypoxia-inducible factor, HIF1, and the cause for this increase in transcript levels in response to hypoxia is not yet known. However, synthesis of heme depends on oxygen (Lash, 2005), so it may be advantageous for mammalian cells to increase their uptake of heme from the gut in response to hypoxia.

Although the authors detected relatively small changes in *HCP1* mRNA levels, the subcellular distribution of HCP1 changes dramatically depending on the iron status of the animal. HCP1 resides on the apical membrane of intestinal epithelial cells in iron-deficient mice, whereas it resides in internal cellular compartments in iron-replete animals (see Figure 1). Moreover, HCP1 redistributes from the apical membrane to internal membrane compartments when iron-deficient animals receive a bolus of iron. The redistribution of transporters from internal compartments to the plasma membrane of polarized epithelial cells has been observed for the Menkes protein, a copper transporter that relocates to the basolateral membrane when animals are copper deficient, thereby promoting entry of copper into the bloodstream. Several motifs important in trafficking of transporters have been recently identified in the Menkes protein, including a dileucine motif and a signal for targeting this copper transporter to the basolateral membrane of epithelial cells (Greenough et al., 2004). Regulated recycling of transporter proteins between the plasma membrane and internal compartments is an increasingly common observation in a variety of experimental systems ranging from yeast to nerve cells (Kim et al., 2005; Ribeiro et al., 2005). More work is needed to identify the signals that mediate HCP1 trafficking to

internal compartments. Colocalization of HCP1 with endosomal markers such as Rab 5 or Rab 11 or with other markers may help to characterize the internal compartments in which HCP1 resides.

Once the intracellular compartments to which HCP1 relocates in iron-rich animals have been characterized, the larger question will be how is such trafficking regulated? In iron-rich animals, the iron transporter ferroportin in the basolateral membrane of gut epithelial cells moves to an intracellular location (the lysosomes) after binding to hepcidin, a peptide hormone synthesized in the liver (Nemeth et al., 2004). However, when released from the liver into the bloodstream, hepcidin most likely cannot directly access the apical membrane or internal compartments of gut epithelial cells, suggesting a more complicated mechanism for regulation of HCP1 trafficking. Perhaps the time has come when protein trafficking researchers will recognize that identification of targeting motifs, intracellular compartments, and the regulation of iron and heme transporters in polarized epithelial cells represents an important frontier. Similarly, metallobiology researchers should pay attention to the changes in subcellular locations of transporters such as HCP1 that accompany alterations in physiological stimuli and should attempt to understand how this movement of transporters is regulated.

Tracey A. Rouault
Cell Biology and Metabolism Branch
NICHD
Bethesda, Maryland 20892

Selected Reading

Abboud, S., and Haile, D.J. (2000). J. Biol. Chem. *275*, 19906–19912.

Abramson, J., Kaback, H.R., and Iwata, S. (2004). Curr. Opin. Struct. Biol. *14*, 413–419.

Andrews, N.C. (2000). Nat. Rev. Genet. *1*, 208–217.

Donovan, A., Brownlie, A., Zhou, Y., Shepard, J., Pratt, S.J., Moynihan, J., Paw, B.H., Drejer, A., Barut, B., Zapata, A., et al. (2000). Nature *403*, 776–781.

Greenough, M., Pase, L., Voskoboinik, I., Petris, M.J., O'Brien, A.W., and Camakaris, J. (2004). Am. J. Physiol. Cell Physiol. *287*, C1463–C1471.

Gunshin, H., Mackenzie, B., Berger, U.V., Gunshin, Y., Romero, M.F., Boron, W.F., Nussberger, S., Gollan, J.L., and Hediger, M.A. (1997). Nature *388*, 482–488.

Kim, Y., Lampert, S.M., and Philpott, C.C. (2005). EMBO J. *24*, 952–962.

Lash, T.D. (2005). Bioorg. Med. Chem. Lett. Published online July 29, 2005. 10.1016/j.bmcl.2005.07/010.

McKie, A.T., Marciani, P., Rolfs, A., Brennan, K., Wehr, K., Barrow, D., Miret, S., Bomford, A., Peters, T.J., Farzaneh, F., et al. (2000). Mol. Cell *5*, 299–309.

McKie, A.T., Barrow, D., Latunde-Dada, G.O., Rolfs, A., Sager, G., Mudaly, E., Mudaly, M., Richardson, C., Barlow, D., Bomford, A., et al. (2001). Science *291*, 1755–1759.

Nemeth, E., Tuttle, M.S., Powelson, J., Vaughn, M.B., Donovan, A., Ward, D.M., Ganz, T., and Kaplan, J. (2004). Science *306*, 2090–2093.

Ponka, P. (2002). Semin. Hematol. *39*, 249–262.

Ribeiro, F.M., Black, S.A., Cregan, S.P., Prado, V.F., Prado, M.A., Rylett, R.J., and Ferguson, S.S. (2005). J. Neurochem. *94*, 86–96.

Shayeghi, M., Latunde-Dada, G., Oakhill, J.S., Takeuchi, K., Laftah, A., Khan, Y., Warley, A., Halliday, N., McCann, F., Hider, R.C., et al. (2005). Cell *122*, this issue, 789–801.

Wheby, M.S., Suttle, G.E., and Ford, K.T. (1970). Gastroenterology *58*, 647–654.

DOI 10.1016/j.cell.2005.08.027